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STUDIES ON THE ALKALOIDS OF CEPHALOTAXUS
VII. STRUCTURES AND SEMI-SYNTHESIS OF TWO NEW ANTICANCER HARRINGTONINE ALKALOIDS

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Abstract. Two new anticancer alkaloids were isolated from the weak alkali from *Cephalotaxus fortunei* Hook f., namely neoharringtonine (1) and anhydroharringtonine (2). Two known alkaloids were also isolated for the first time from this plant: deoxyharringtonine (3) and isoharringtonine (4).

Keywords: Cephalotaxus; Cephalotaxine alkaloid; Neoharringtonine; Anhydroharringtonine

This paper reports on 9 cephalotaxine alkaloids isolated from the weakly alkaline part of *Cephalotaxus fortunei* Hook f., of which two are new alkaloids, namely neoharringtonine (1) and anhydroharringtonine (2). Their structures were confirmed through spectroscopy and semi-synthesis. In vitro tests showed that at a concentration of 1 μg/ml their inhibition rate of white mice leukaemia P 388 was equivalent to that of harringtonine (8). The remaining alkaloids were the known anhydroharringtonine (3), isoharringtonine (4), isocephalotaxinone (5), (+) acetylcephalotaxine (6), cephalotaxine (7), harringtonine (8) and, homoharringtonine (9). 4 and 3 were isolated from this plant for the first time.

Structure of Neoharringtonine (1)

1 was a yellow oily substance, $[\alpha]_D$-101° (c 0.25 $CHCl_3$), with a molecular weight of 535.2187 (HRMS), and the molecular formula $C_{30}H_{33}NO_8$. IR showed it to have an ester group (1745 cm^{-1}) and a hydroxyl group (3520 cm^{-1}). MS m/z 315, 314, 298 (100), 284, 282, 266, 150. By comparing the hydrogen spectrum of 1 with cephalotaxine (7), it is known that 1

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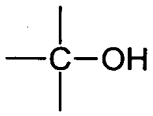
was an ester compound containing the cephalotaxine parent body [1], and that for 1, the C_3-H δ ppm shifted from the 4.70 position to 5.96, showing that the C_3-position hydroxyl group had already been acylated. δ ppm 7.17 (5H, m) and 2.67 (2H, s) indicated that there was a benzyl group. Also, with 2.67 illumination, a NOB effect was confirmed at 7.17. δ ppm 3.55 (3H, s) was a methoxy group signal. δ ppm 2.27, 1.94 (2H, dd, J = 16 Hz) indicated that there was a methylene group on the chiral carbon. A comparison of the hydrogen spectra of 1 and deoxyharringtonine (3), showed δ ppm 3.55, 2.27, 1.94, which is the proton signal of the methyl ethanoate group attached to the chiral carbon. Removing the cephalotaxine parent body (7) and a carbonyl group, one benzyl group and a methyl ethanoate group ($-CH_2COOCH_3$), from the molecular formula of 1 leaves only CHO, and as the hydrogen spectrum had no secondary methyl group proton signal, and the IR indicated a hydroxyl group, the CHO arrangement is most appropriately  . It can be postulated by summarizing the above that the structure is as shown in 1. The CD curve in 1 was 288 (Δε-0.20), 248 (Δε-0.28) and 206 (Δε -5.98) nm. Its ^1L_b and ^1B bands both showed a negative Cotton effect, and it is considered that the stereo form of the parent body of 1 is the same as that for cephalotaxine (7), namely 4*S*, 5*R* [2]. As $J_{3,4}$ = 9.6 Hz, C_3 was the *S* form [3], from which it is determined that the stereo arrangement of the 1 parent body was 3*S*, 4*S*, 5*R*. It was discovered from a comparison of the hydrogen spectra of 1 and deoxyharringtonine (3) and its C_2 epimer (3'), that the chemical shift values of C_3-H, C_{14}-H, CH_3OCO-, C_3-H for 1 and 3 were almost the same, while the differences between the abovementioned proton chemical shift values between 1 and 3 were very large. It can be deduced from this that the absolute form of the acyl branch chain C_2 of 1 and 3 were both *R* form [4], and that the absolute form of 1 was 3*S*, 4*S*, 5*R* and 2'*R*.

Structure of Anhydroharringtonine (2)

2 was a yellow oily substance; $[\alpha]_D^{18}$ -94° (c 0.45, $CHCl_3$), with a molecular weight of 513. 2402 (HRMS), and a molecular formula of $C_{28}H_{35}NO_8$. IR indicated an ester group (1738 cm^{-1}), and MS m/z 315, 314, 298, 284, 267, 266, 150 indicated that 2 was an ester compound containing the cephalotaxine parent body. Comparing the mass spectra and

hydrogen spectra of 2 and harringtonine (8), 2 was lower than 8 by 18 units of mass. As the parent bodies of 2 and 8 were exactly the same, this shows that the acyl group branch chain of 2 was 18 units of mass less than the acyl group branch chain of 8, and it can be deduced that the two hydroxyl groups in the branch chain of 8 were dehydrated and cyclized to form a tetrahydrofuran ring or an alkene chain. As the IR spectrum of 2 showed no hydroxyl group absorption peak, and its proton signal met the previous postulation, it is deduced that the structure was as in 2. The CD curve in 2 was 289 ($\Delta\varepsilon$ -0.32), 236.5 ($\Delta\varepsilon$ +0.64) and 203 ($\Delta\varepsilon$ -20.2) nm. Its ^1L_b and ^1B bands both showed a negative Cotton effect. It is deduced that the absolute arrangement of the parent body of 2 was 4*S*, 5*R*, and again according to $J_{3,4}$ = 9 Hz, C_3 was S form. It was discovered by comparing the hydrogen spectra of 2 with harringtonine (8) and its C_2 epimer (8'), that the chemical shift values of C_3-H, C_{14}-H, CH_3OCO-, C_3-H for 2 and 8 were almost the same, while the differences between the abovementioned proton chemical shift values between 2 and 8 were very large [4]. It is deduced from this that the absolute forms of the acyl branch chains C_2 of 2 and 8 were both *R* form, and that the absolute form of 2 was 3*S*, 4*S*, 5*R* and 2'*R*.

Semi-Synthesis of Neoharringtonine (1) and 2'-Epineoharringtonine (1')

Cephalotaxine (7) and phenyloxopropanoyl chloride were allowed to react, forming α-ketoyl cephalotaxine, the latter undergoing a Reformatsky reaction to form 1 and its C_2' epimer (1') [5], with an overall yield of 27%.

[Equation]

Semi-Synthesis of Anhydroharringtonine (2)

Harringtonine (8) was catalytically dehydrated using *p*-toluene sulphonic acid to yield 2, with a yield of 93%.

[Equation]

Experimental Components

The melting point was measured with a Kofler micro-melting point instrument, which had not been calibrated. Rotation was measured with a Jasco Dip-181 polarimeter. A Perkin-Elmer 599 B instrument was used for IR measurement, LRMS was measured with a MAT-44 instrument, HRMS with a MAT-711 instrument, and ^{1}HNMR with a Bruker Am-400. The silica gel was produced by the Qingdao Marine Chemical Engineering Works, and the developer was iodine vapour or a Dragendorff reagent.

The material source was the Changzhou Jianmin Pharmaceutical Works, and it was the weakly alkaline part of the mother liquor after production of harringtonine. Production by that works was from *Cephalotaxus fortunei* Hook f. plants gathered from Huang Shan.

Alkaloid Separation

73 g of the weak alkali was packed into a column according to the development method and was separated into layers by silica gel (1.6 kg). Gradient elution was carried out with chloroform-methanol, and the mobile fractions were collected. The same liquid fractions were combined according to thin layer examination, and they were then concentrated and subjected to further thin layer chromatography to prepare the separation (developed with cyclohexane-ethyl ethanoate-diethylamine 9 : 1 : 1). The corresponding colour bands were scraped off under fluorescent light, and the single product obtained was purified by silica gel column chromatography or recrystallization.

Identification

Neoharringtonine (1) was an oily substance, $[\alpha]_D$-101° (c 0.25 $CHCl_3$). IR (KBr) cm^{-1} 3520, 1745, 1655, 1505, 1490, 1370, 1275, 1225, 1120, 1035, 930, 750, 700. EIMS m/z 535 (M^+), 504, 315, 314, 299, 298 (100), 284, 282, 266, 150. ^{1}HNMR (CD_3COCD_3) δ ppm 7.21 (5H, m, ph-H), 6.71 (1H, s, C_{17}-H), 6.61 (1H, s, C_{14}-H), 5.92 (1H, d, J = 9.6 Hz, C_3-H), 5.89 (2H, d, -OCH_2O-), 5.35 (1H, s, C_1-H), 3.98 (1H, d, J = 9.6 Hz, C_4-H), 3.78 (3H, s, -OCH_3), 3.50 (3H, s, -$COOCH_3$), 2.16, 1.93 (2H, dd, J = 16 Hz, C_3-H), 2.72 (2H, s, $PhCH_2$). ^{1}HNMR ($CDCl_3$) δ ppm 7.17 (5H, m, Ph-H), 6.65 (1H, s, C_{17}-H), 6.56 (1H, s, C_{14}-H), 5.96 (1H, d, J = 9.4 Hz, C_3-H), 5.87 (2H, d, -OCH_2O-), 5.05 (1H, s, C_1-H), 3.80 (1H, d, C_4-H), 3.76 (3H, s, -OCH_3), 3.55 (3H, s, -$COOCH_3$), 2.27, 1.94 (2H, dd, J = 16 Hz, C_3-H), 2.67 (2H, s, $PhCH_2$). HRMS

535.2178 ($C_{30}H_{33}NO_8$), 504.1992 ($C_{29}H_{30}NO_7$), 298.1144 ($C_{18}H_{30}NO_3$), 284.1050 ($C_{17}H_{18}NO_3$), 282.1159 ($C_{17}H_{16}NO_4$), 266.1130 ($C_{17}H_{16}NO_2$), 150.0915 ($C_9H_{12}NO$).

Anhydroharringtonine (2) was an oily substance; $[\alpha]_D^{18}$ -94° (c 0.445, $CHCl_3$), IR (KBr) cm^{-1} 1738, 1650, 1500, 1485, 1455, 1365, 1270, 1220, 1035, 930. EIMS m/z 513 (M^+),482, 315, 314, 299, 298 (100), 284, 282, 268, 267, 266, 228, 150. ^{1}HNMR (CD_3COCD_3) δ ppm 6.63 (1H, s, C_{17}-H), 6.58 (1H, s, C_{14}-H), 5.90 (2H, d, -OCH_2O-), 5.85 (1H, d, J = 9.6 Hz, C_3-H), 5.25 (1H, s, C_1-H), 3.90 (1H, d, J = 9.6 Hz, C_4-H), 3.72 (3H, s, -OCH_3), 3.51 (3H, s, -$COOCH_3$), 2.31 (2H, dd, J = 15.6 Hz, C_3-H), 1.18 (3H, s, -CH_3), 1.09 (3H, s, -CH_3). ^{1}HNMR ($CDCl_3$) δ ppm 6.57 (1H, s, C_{14}-H), 5.87 (2H, d, -OCH_2O-), 5.87 (1H, d, J = 9.6 Hz, C_3-H), 5.00 (1H, s, C_1-H), 3.70 (3H, s, -OCH_3), 3.69 (1H, d, C_4-H), 3.56 (3H, s, -$COOCH_3$), 2.30 (2H, dd, C_3-H), 1.20, 1.12 (6H, CH_3-C-CH_3). HRMS 513.2402 (11) $C_{28}H_{35}NO_8$), 314.1369 (9) ($C_{18}H_{20}NO_4$), 298.1466 (100) ($C_{18}H_{20}NO_3$), 284.1290 ($C_{17}H_{18}NO_3$), 282.1151 (10) ($C_{17}H_{16}NO_3$), 266.1183 (10) ($C_{17}H_{16}NO_2$), 150.0935 (6) ($C_9H_{12}NO$).

The rotation, infrared, nuclear magnetic and mass spectroscopy data of the other known alkaloids were all the same as reported in the literature [6-10].

Semi-Synthesis of Neoharringtonine (1) and 2'- epineoharringtonine (1')

118 mg of dry sodium phenyl-α-oxopropanoate was placed in 1 ml of dry benzene, and 0.05 ml of dry pyridine was added dropwise. This was shaken until uniform and cooled until the benzene set. 0.15 ml of freshly distilled oxalyl chloride was slowly added dropwise and agitated overnight at room temperature. The solvent was removed under reduced pressure and the oxalyl chloride residue was placed in 0.5 ml of dry dichloromethane. It was cooled on an iced saline bath, and a solution made up of 91 mg of cephalotaxine (7), 0.5 ml of dry dichloromethane and 0.4 ml of pyridine was added dropwise under nitrogen protection, and it was again agitated overnight at room temperature. The reaction liquid was washed twice each in 10% Na_2CO_3 and saturated saline and dried with anhydrous Na_2SO_4. The solvent was evaporated off to yield 120 mg of α-ketoyl harringtonine, with a yield of 90%. 20 mg was taken and separated by column chromatography with 2 g of silica gel, and eluted with chloroform-methanol (100 : 1). It was discovered that the product had decomposed and

yielded only 2 mg of the pure product as a solid. IR (KBr) cm^{-1} 3420, 1775, 1720, 1655, 1505, 1490, 1375, 1260, 1220, 1035, 930. EIMS m/z 461 (M^+), 430, 314, 299, 298, 282, 256, 150, 149.

In accordance with the method in the literature [5], 100 mg of α-ketoyl harringtonine was taken and subjected to a Reformatsky equation with methyl bromoethanoate. The crude product was purified by column chromatography using 10 g of silica gel to yield 35 mg of a pale yellow solid, with a yield of 30%. It was purified a second time by column chromatography using 4 g of silica gel, to yield 10 mg of a mixture of neoharringtonine (1) and 2'-epineoharringtonine (1'), as a colourless oily substance $[\alpha]_D$-85° (c 0.66, $CHCl_3$). IR (KBr) cm^{-1} 3515, 1745, 1655, 1505, 1490, 1365, 1275, 1225, 1120, 1038, 930, 755, 700. EIMS m/z 535 (M^+), 504, 315, 314, 299, 298 (100), 284, 282, 266, 150. ^{1}HNMR (CD_3COCD_3) δ ppm 7.21 (5H, m, Ph-H), 6.70 (1H, s, C_{17}-H), 6.59 (1H, s, C_{14}-H), 5.91 (1H, d, J = 9.9, C_1-H), 5.89 (2H, d, -OCH_2O-), 5.30 (1H, s, C_1-H), 3.92 (1H, d, C_4-H), 3.77 (3H, s, -OCH_3), 3.51 (3H, s, -$COOCH_3$), 2.74 (2H, s, $PhCH_2$), 2.16, 1.93 (2H, dd, J = 16, C_3-H). The above hydrogen spectral data only gave a proton signal of C_2 as *R* form.

Semi-Synthesis of Anhydroharringtonine (2)

50 mg of harringtonine (8) was dissolved in 2 ml of dry benzene. 15 mg of *p*-toluene sulphonic acid was added, and refluxing was carried out to remove the water. After 2 h, the starting material points in thin layer examination disappeared, and the reaction liquid was washed twice in succession with 10% Na_2CO_3 and saturated saline respectively. The solvent was evaporated off and purified by column chromatography using 5 g of [illegible] (10 - 40 μ). This yielded 45 mg of an oily substance with a yield of 93%. After crystallization in cyclohexane, recrystallization was carried out in propanone-ether to yield pale yellow needle-shaped crystals, mp 138 - 139°C. $[\alpha]_D^{14}$ -91° (c 0.35, $CHCl_3$). IR (KBr) cm^{-1} 1740, 1655, 1505, 1490, 1460, 1370, 1270, 1225, 1035, 930. EIMS m/z 513 (M^+), 482, 315, 314, 300, 299, 298 (100), 284, 282, 268, 267, 266, 228, 150. ^{1}HNMR (CD_3COCD_3) δ ppm 6.60 (1H, s, C_{17}-H), 6.54 (1H, s, C_{14}-H), 5.87 (2H, d, -OCH_2O-), 5.80 (1H, d, J = 9.6 Hz, C_3-H), 5.20 (1H, s, C_1-H), 3.84 (1H, d, J = 9.6 Hz, C_4-H), 3.68 (3H, s, -OCH_3), 3.48 (3H, s, -$COOCH_3$), 2.29 (2H, dd, J = 16 Hz, C_3-H), 1.15 (3H, s, -CH_3), 1.07 (3H, s, -CH_3).

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